Thankfully, Spring has been in the air for a few weeks now and we can witness the remarkable and exquisite growth of nature all around us. In the same way, we as a department are experiencing growth in several areas. I would like to welcome our new Dean, Dr. Robert T. Means, Jr., MD., whose support for our department is evident already. We are well on our way to recruiting three new Faculty and the Recruitment Committee, Mary Lou Hawk, and I have spent countless hours evaluating and considering applicants. I am grateful for everyone’s efforts and am very pleased with the caliber of the candidates who we will interview over the next few months. Drs. Ferguson and Ardell announced their retirement after many years of excellent service to the department. We are grateful for everything they have done and will wish them well at their respective farewell receptions. Their retirements mean that more change will come our way in the form of another round of recruitment. We have witnessed May graduation, including that of Medical and BMS Graduate students, and are rightly proud of their accomplishments and the role of our superb teaching core of Faculty and Staff in their education. Congratulations to several of these Faculty who have received prestigious teaching awards. Well-deserved! The Course Directors, individually and as a group, as well as those serving on various Quillen committees are continuing to improve the curriculum. Our department has also invested in equipment to help foster growth of our research. We purchased two Agilent 2200 TapeStation systems, which modernized our molecular biology capabilities, and a departmental -80 freezer which serves as a backup, and arrived just in time for its intended use, saving precious samples from a malfunctioning freezer. I am very grateful to Dr. Don Hoover and Rolf Fritz for organizing a successful demonstration of state-of-the-art confocal microscopes by three potential vendors. We are looking forward to purchasing such a system for our Microscopy Core and the great advances in our science we will be able to make because of it. Our research education has grown even more with the start of a new DBMS Journal club, and I am particularly thankful to Dr. Sharon Campbell who volunteered to direct it. On a personal note, my family has been blessed with two new children through adoption and we expect them to come home in the summer.

So, yes I would say change is in the air, the really good kind.
DONALD “DON” A. FERGUSON, JR., Ph.D., PROFESSOR

After more than 35 years of dedicated service
Dr. Donald A. Ferguson, Professor, plans retirement
May 31, 2014

Dr. Don Ferguson considers himself fortunate to have had the unique opportunity to participate in the creation of a microbiology department in the newly established James H. Quillen College of Medicine. He was the first faculty member hired under the supervision of Dr. Dwight Lambe, then Chairman of the Department of Microbiology. He joined the faculty as Assistant Professor in May of 1978. He was appointed to the graduate faculty in 1980, and became tenured in 1984. In 1990, he received promotion to Associate Professor. On July 1, 2010, Don received promotion to Full Professor in the Department of Microbiology.

Prior to joining the College of Medicine faculty, Don was Research Associate in the Anaerobe Laboratory of Virginia Polytechnic Institute and State University, in Blacksburg, Virginia. In 1967, he earned an A.B. in Biology from Clark University, Worcester, Massachusetts. And, in 1974, he earned his Ph.D. from Syracuse University, Syracuse, New York.

He is accredited, with Dr. Dwight Lambe and Dr. William Campbell, with the establishment of a diagnostic microbiology laboratory in the Department of Microbiology in 1979. This laboratory has thence served as a reference laboratory for bacteriology and virology for local hospitals and physicians. In this capacity, Dr. Ferguson attained licensure as a clinical laboratory director in 1981. He served as Co-Director of the laboratory until June 1998, when he assumed the Directorship.

During his tenure with the College of Medicine, he remained a well-funded researcher, which resulted in numerous journal publications. His research involved biochemical characterization of structural proteins and polysaccharides (virulence factors) of anaerobic and microaerophilic bacteria, bacterial taxonomy studies, sero-epidemiology of Campylobacter and Helicobacter pylori infections and molecular biology studies of Helicobacter pylori DNA for the purpose of developing a specific genetic probe for epidemiologic studies. More recently his research has involved sepsis models in mice as a means of studying the effects of glucans, the mechanism of action of C-reactive protein and the innate immune response.

His teaching responsibilities have included lecturing medical students in the areas of immunity to infection, bacterial adherence, and pathogenic bacteriology including anaerobic infections and anti-microbial susceptibility testing. He also mentored graduate students through Chairing of Graduate Committees as well as serving as Committee Member for several doctoral and master students.

Upon retirement Don plans to spend more time pursuing his personal interests. He enjoys music but his passion is classical music. He is somewhat of a master gardener and especially enjoys growing roses. Hailing from the New England area, he developed an interest at an early age in deep sea fishing which he still enjoys today.

We sincerely appreciate Don for his many years of dedicated service and wish him the very best throughout his retirement years.
The Department Of Biomedical Sciences
Cordially Invites You To An Informal Drop-By Reception For
Dr. Donald Ferguson
In Honor Of His Service To East Tennessee State University And The Quillen College Of Medicine
Thursday, May 29, 2014
2:00 p.m. – 4:00 p.m.
Stanton-Gerber Hall
Ground Floor Mezzanine
2014 CADUCEUS NOMINEES—DBMS

**Course of the Year—M1**
- Cell/Tissue Biology
- Cellular/Molecular Medicine
- Gross Anatomy/Embryology
- Medical Physiology

**Course of the Year—M2**
- Clinical Neuroscience
- Immunology
- Microbiology
- Pathology
- Pharmacology

**Professor of the Year—M1**
- Jason Moore
- Phil Musich
- Mitch Robinson
- Brian Rowe
- Antonio Rusinol
- Tom Sadler
- Krishna Singh
- Doug Thewke

**Professor of the Year—M2**
- Michelle Duffourc
- Kenneth Ferslew
- Jennifer Hall
- Russell Hayman
- Rob Schoborg
- John Kelley Smith

**Outstanding Staff**
- Rob Becker
- Jerry Keplinger
- Tonya Ward

2014 CADUCEUS CEREMONY RESULTS

**M1 – Outstanding Course of the Year:** Awarded to the basic science course which 1st year students regard as the best educational experience including curriculum, content, and professor interaction with students.

*Medical Human Gross Anatomy (Dr. Tom Kwasigroch, Course Director)*

**M1 – Professor of the Year:** Awarded by the 1st year students to basic science faculty demonstrating exemplary professionalism, mentorship, and scholarship.

*Dr. Tom Kwasigroch*

**M2 – Outstanding Course of the Year:** Awarded to the basic science course which 2nd year students regard as the best educational experience including curriculum, content, and professor interaction with students.

*Microbiology (Dr. Russ Hayman, Course Director)*

**M2 – Professor of the Year:** Awarded by the 1st year students to basic science faculty demonstrating exemplary professionalism, mentorship, and scholarship.

*Dr. Rob Schoborg*
SCARLET SASH SOCIETY AWARDS
ANNOUNCED BY THE GRADUATING CLASS OF 2014

The Scarlet Sash Society Award is given to only 10 faculty members per year. The Awardees in Biomedical Sciences for 2013-2014 are:

Dr. Michelle Duffourc
Dr. Tom Kwasigroch
Dr. Paul Monaco
Dr. Robert Schoborg

These honors are further evidence of the department’s commitment to maintaining excellence in teaching and in helping the Quillen College of Medicine remain one of the top medical schools in the country.

TEACHING EFFORTS OF DBMS FACULTY RECEIVE HIGH RECOGNITION BY STUDENTS

The Medical Microbiology team led by Course Director, Dr. Russ Hayman, achieved an outstanding overall score on the “MY OVERALL EVALUATION OF THIS COURSE IS:" of 4.90/5.0 in the student survey. This team consists of Drs. Hayman, Kruppa, Hall, Schoborg, Ferguson, with support of Jerry Keplinger, Diana Toler and Crystal Maupin. The students noted that this team has a commitment to their well-being and education, and was characterized by a strong cohesiveness as a faculty. Way to go!

DR. HOSSLER’S BEAUTIFUL NEW ATLAS GETS BIG PROMOTION AND DISPLAY AT THE EXPERIMENTAL BIOLOGY MEETING AT SAN DIEGO, CA

WELCOME … CHIHARU LOVINS

Chiharu Lovins has joined the Department as Director of Research in Dr. Hagg’s lab. She was previously employed in the laboratory of Dr. Theo Hagg at the University of Kentucky. Chiharu completed her Bachelor’s Degree in Biology with emphasis on anatomy and physiology from California State University, Long Beach, CA. She holds a Master’s Degree in Biochemistry from SUNY Upstate Medical University, Syracuse, NY. Chiharu will continue to provide support to Dr. Hagg’s research activities.

Medical Students Express Appreciation to Faculty

Dear Biomedical Sciences Faculty,

“Thank you so much for all that you have done for us over these past four years! You have taught us hundreds of lectures, toiled over exam schedules, handouts, and power points, calculated and re-calculated countless exams, and the list goes on. Thank you for all of these things, as well as all that you have done that we will never know because you did so well. We could have never made it without you.”

All of our best,
QCOM Class of 2014
FORMER PHYSIOLOGY STUDENT, DR. DEIDRA J. H. MOUNTAIN, IS INVITED GUEST SPEAKER

Dr. Deidra J. H. Mountain, Ph.D., Associate Professor, Department of Surgery, and Scientific Director, Vascular Research Laboratory, University of Tennessee Graduate School of Medicine, Knoxville, TN, has been invited as the Guest Speaker for the Biomedical Graduate Program Spring Banquet. The banquet was held on May 8, 2014, at the Old Quarters in Jonesborough, Tennessee. Dr. Mountain will also be conducting a seminar in conjunction with the Biomedical Science Graduate Program and the Department of Biomedical Sciences, on May 8th at 11:30 A.M. in the Small Auditorium in Stanton-Gerber Hall. The subject of her seminar will be, "Combating Vascular Disease Through Molecular and Genetic Targets."

Dr. Mountain is a former graduate from the Quillen College of Medicine Biomedical Sciences Graduate Program. Deidra was a graduate student in Dr. Krishna Singh’s cardiovascular research laboratory. She completed the requirements for her doctoral degree with a concentration in Physiology in December 2006. Upon graduation, Deidra joined the Vascular Research Laboratory at the University of Tennessee Graduate School of Medicine as a Postdoctoral Research Associate. In 2008, she was appointed as Assistant Professor in the Division of Vascular and Transplant Surgery, Department of Surgery, and promoted to Associate Professor in 2012. She also currently serves as Associate Professor in the Core Faculty of the Institute of Biomedical Engineering at the University of Tennessee-Knoxville, and in the Department of Comparative and Experimental Medicine at the University of Tennessee College of Veterinary Medicine.

In 2010 Dr. Mountain was appointed the Scientific Director of Vascular Research Laboratory, and now oversees and manages all research activities of the lab. The Vascular Research Laboratory is a basic and translational research facility dedicated to the study of peripheral vascular disease and the identification of critical rate-limiting steps where targeted interventions could prevent or hinder development of vascular pathologies. Their mission is to take a collaborative approach to translational research of peripheral vascular pathology with scientists and physicians working closely together on clinically significant research endeavors. This provides a distinct advantage in developing progressive approaches to solving clinically relevant problems. The laboratory focuses on research into the molecular and cellular mechanisms of vascular restenosis, intimal hyperplasia development, and vessel graft failure. Additionally, significant emphasis is placed on understanding the hormonal regulation of vascular wall structure and remodeling post-injury (specifically the influences of hormone replacement therapy in women and androgen deficiency in men) and the development of translational approaches to gene therapy in the prevention of vascular disease.
GRANT AWARDS—CONGRATULATIONS!

**Funding Agency:** National Institutes of Health

**Grant Number:** 2R01AG029493-06A1

**Principal Investigator(s):** Theo Hagg, MD, PhD

**Project Title:** Targeting CNTF to increase adult forebrain neurogenesis.

**Project Funding Period:** 04/15/2014 – 03/31/2019

**Total Amount of Award:** $1,496,500

[Director of Research, Chiharu Lovins provided preliminary data for this].

**Project Narrative.** This grant examines the role of our newly discovered integrin signaling pathway in reducing CNTF, one of the important molecules involved in stem cell proliferation and new cell production in the normal adult brain and after stroke. We previously found that stroke causes an increase in CNTF and this so-called neurogenesis, and that we can increase both by using pharmacological drugs which block this integrin pathway. Using both genetic approaches and pharmacological drugs, we expect to identify new treatments to maximize neurogenesis for replacing lost cells or protecting surviving ones that will hopefully extend beyond stroke to treatments of other neurological disease.

GRANT AWARDS—CONGRATULATIONS!

**Funding Agency:** Department of Veterans Affairs

**PD/PI:** Dr Krishna Singh; Co-PI/Co-PD: Dr Mahipal Singh; Consultant: Dr Chuanfu Li

**Project Title:** Osteopontin: role in myocyte apoptosis and myocardial function

**Award Type:** Merit Review

**Project Funding Period:** 07/01/2014 – 06/30/2018

**Annual Direct cost:** $241,364.00

**Total Direct Cost:** $965,456.00

**Project Narrative:** Heart failure represents a major cause of morbidity and mortality among veterans. Cardiac myocyte loss due to apoptosis plays a significant role in the progression of heart failure. Expression of osteopontin (OPN; a matricellular protein) increases markedly in the heart under a variety of pathophysiological conditions. Increased OPN expression, specifically in myocytes, associates with increased myocyte apoptosis and myocardial dysfunction. The objectives of this study are directed towards understanding the intracellular signals by which increased OPN expression induces cardiac myocyte apoptosis and myocardial dysfunction. The proposed studies investigating the intracellular signaling pathway involved in OPN-stimulated myocyte apoptosis and myocardial dysfunction may uncover novel therapeutic strategies for the treatment of heart failure.
This book not only represents over 900+ pages of scanning and transmission electron microscope images of the major systems of the body. But, more importantly, it represents a lifetime of scientific work for Dr. Fred Hossler.

Dr. Moises Serrano is a Clinical Cytogenetics Fellow at University of Utah. This is an extremely competitive program. Most of the programs in the nation admit only one fellow a year. You can go to the following link to find more about him and the program: http://www.path.utah.edu/education/fellowships/fellows.

Moises also published last year a paper in Oncogene, one of the top cancer research journals in the world. Serrano MA, Li Z, Dangeti M, Musich PR, Patrick S, Roginskaya M, Cartwright B, Zou Y. (2013) “DNA-PK, ATM and ATR collaboratively regulate p53-RPA interaction to facilitate homologous recombination DNA repair” Oncogene. 32, 2452-62

Moises received his Ph.D. in 2013, and worked under the direction of Dr. You Zou.

In Appreciation…

Dr. Fred Hossler served as Judge at the Upper East Tennessee Science Fair, Saturday, March 22, 2014. The fair was host to over 250 exhibits from 40 area elementary and middle schools. “Without the generous gifts of time and expertise which he donated to our fair, the task of judging all those projects would have been overwhelming.”

- Leonard Robertson, Vice-President UETSF

Dr. Alok Agrawal, Professor, has all rights to be proud his son, Devanshu. Devanshu has been recognized as Outstanding Student in both the Department of Mathematics and Statistics and the Department of Physics and Astronomy. He has also been recognized as an Honors-in-Discipline Student in mathematics, and recipient of the Stanton Honors Scholars Scholarship. Devanshu has recently been the featured student on ETSU’s main web page.

Devanashu is no ordinary student. Becoming blind at an early age, he did not let his disability deter him from setting high goals. In May 2014, he graduated from ETSU Summa Cum Laude with a double major in math and physics. He plans to continue his education and will be entering into school at ETSU this Fall to begin his graduate studies. He has received offers from Vanderbilt, Indiana University-Bloomington, and Georgia Tech. His long term goals are to pursue a Ph.D. and a career in Academia—to become a Professor, Teacher, and Researcher.

Devanashu interests are diverse and go far beyond math and sciences. He also enjoys the arts and in particularly enjoys all forms of literature—poetry, fiction, fantasy, and philosophy.

We congratulate Devanashu Agrawal on his many accomplishments and wish him the very best in his future educational and career endeavors.

In Memory

Adjunct Professor of Anatomy and Cell Biology, Dr. Frederick H. Kasten, passes away April 14, 2014, at the age of 87. In August 2009, he was chosen Scholar-in-Residence at the Sherrod Library at ETSU.
RECENT BOOKS/BOOK CHAPTERS


[The Ultrastructure Atlas of Human Tissue offers a unique and comprehensive look at the structure and function of tissues at the sub-cellular and molecular level, an important perspective in the understanding and combating disease.]

RECENT JOURNAL PUBLICATIONS


SCIENTIFIC MEETINGS/PRESENTATIONS

Dr. Meng-Yang Zhu, Professor, has been invited as a seminar speaker at the Zhongshan Ophthalmic Center and Department of Pharmacology, Sun Yat-sen University, Guangzhou, China, March 24-28, 2014. His topics were: "Effects of chronic social defeat and glucocorticoids on norepinephrine transporter expression and underlying molecular mechanisms," and "Transcription factors and noradrenergic phenotypes."

Dr. Alok Agrawal, Professor, presented a seminar in the "School of Medicine Spring Seminar Series" in the "Department of Pathology, Microbiology and Immunology", University of South Carolina School of Medicine, Columbia, SC. The seminar is on April 28, 2014, and the title of the seminar is "C-reactive protein in pneumococcal infection.

COMMITTEE ASSIGNMENTS

Dr Krishna Singh, Professor, served on the Cardiac biology regulation (BSci 5; Basic and clinical-translational science) grant peer-review committee, American Heart Association (meeting date March 31, 2014; teleconferencing).

Dr. Gregory Ordway, Professor, served on the Research Grants Committee, American Foundation for Suicide Prevention (meeting date April 2014, New York.)

Dr. Phil Musich, Professor, has been appointed to serve as a member on the Ad Hoc Award Committee for Research Grant Awards, ETSU, School of Graduate Studies.
SEMINAR NEWS...

One of the goals of the Department is to strengthen our seminar program. As Chair of program, it is a major challenge to seek out researchers, both on the international as well as national levels, to be a part of this program.

We acknowledge with many thanks and sincere appreciation Dr. Mike Kruppa for his willingness to serve as Chair and Coordinator of the external Seminar program for the last two years. Through his efforts we have definitely strengthened our seminar program and thus strengthened our educational foundation in the Department and College. Effective July 1, 2014, Dr. Yue Zou will resume responsibility as standing Chair for a two-year term. We appreciate Dr. Zou’s willingness to accept this responsibility and look forward to continued quality educational seminars under his guidance.

Additionally, Dr. Rob Schoborg will be chairing the newly organized Internal Seminar Program. Designed to provide an opportunity for faculty to share their research interests.

Ms. Cindy Canter, who has been serving as staff support for the seminar program will continue to serve in this capacity both programs.

RECENT SEMINARS...

♦ Dr. Romain Guinamard, Professor, Université de Caen Basse-Normandie, France, presented a seminar on March 31, 2014. Title of Seminar: “The TRPM4 ion channel, a new actor in cardiac activity and its disturbances.”

♦ Dr. John McMichael, Ph.D., President & CEO, Beech Tree Labs, Inc., Delanson, NY, presented a seminar on April 11, 2014. Title of Seminar: “RMS a platform for Developing New Therapeutic Agents.”

♦ Dr. Trevor Archer, Professor, Department of Psychology, University of Gothenburg, Sweden, Department of Psychology, presented a seminar on April 14, 2014. Title of Seminar: “Physical Exercise Influences Parkinsonism in a Laboratory Model.”

Dr. Tom Kwasigroch had the distinct honor of being selected to assist in the hooding portion of the Quillen College of Medicine Commencement Ceremony held on May 9, 2014. He was one of only two faculty selected to hood the Medical Graduates.

Dr. Gregory Ordway, Professor, served as Distinguished Faculty Marshall
Dr. Krishna Singh, Professor, served as Distinguished Faculty Coordinator for the College of Medicine.
Christopher Ray Daniels

Candidate for the Degree of Doctor of Philosophy
with a concentration in Physiology
Department of Biomedical Sciences
March 21, 2014

Dissertation Abstract

Extracellular Ubiquitin: Role in Cardiac Myocyte Apoptosis and Myocardial Remodeling

Activation of sympathetic nervous system is a key component of myocardial remodeling which generally occurs following ischemia/reperfusion (I/R) injury and myocardial infarction. It induces cardiac myocyte apoptosis and myocardial fibrosis, leading to myocardial dysfunction. Intracellular ubiquitin (UB) regulates protein turnover by the UB-proteosome pathway. The biological functions of extracellular UB in the heart remain largely unexplored. Previously, our lab has shown that β-adrenergic receptor (β-AR) stimulation increases extracellular UB levels, and extracellular UB inhibits β-AR-stimulated apoptosis in adult rat ventricular myocytes (ARVMs).

This study explores the role of extracellular UB in myocyte apoptosis, fibroblast phenotype and function, and myocardial remodeling following β-AR stimulation and I/R injury. First, left ventricular (LV) structural and functional remodeling was studied 7 days after chronic β-AR-stimulation in the presence or absence of UB infusion. Echocardiographic analyses showed UB infusion decreases β-AR-stimulated increases in percent fractional shortening and ejection fraction. It decreased cardiac myocyte apoptosis and myocardial fibrosis. UB activated Akt, and inhibition of Akt inhibited β-AR-stimulated increases in matrix metalloproteinase-2 expression.

Second, using cardiac fibroblasts, we provide evidence that extracellular UB interacts with the cell surface and co-immunoprecipitates with CXCR4. UB treatment increased expression of α-smooth muscle actin (myofibroblast marker), and induced rearrangement of actin into stress fibers. It inhibited lamellipodia and filopodia formation, and cell migration into the wound. Third, using isolated mouse heart and I/R injury as a model, we provide evidence that UB treatment decreases I/R-mediated increases in infarct size. UB treatment improved functional recovery of the heart as measured by increased % LV developed pressure. Activation of proapoptotic proteins, p-STAT-1 and caspase-9, was significantly lower in UB I/R hearts versus I/R alone. In ARVMs, UB treatment decreased simulated I/R-induced apoptosis. It activated Akt (anti-apoptotic kinase) and inhibited activation of GSK-3β (pro-apoptotic kinase). It decreased I/R-induced oxidative stress and protected anoxia-induced mitochondrial polarization. In fibroblast and ARVMs, CXCR4 antagonism negated the effects of UB, while mutated UBs (unable to interact with CXCR4) had no effect. Thus, extracellular UB, most likely acting via CXCR4, modulates myocardial remodeling with effects on heart function, fibroblast phenotype and function and myocyte apoptosis.

Chris has now accepted a position as Associate Director-Clinical labs with Medpace, a global CRO located in Cincinnati, Ohio, effective May 19, 2014. He will be responsible for monitoring the laboratory operations in order to verify that accurate, precise, and medically-reliable data are being generated for clinical trials for all the pharmaceutical companies and biotech companies. Chris completed his graduate training under the supervision of Dr. Krishna Singh. During his appointment to Dr. Singh’s lab, his research has focused upon studying cardiac remodeling after injury of the heart. He is originally from Cincinnati Ohio, and he is excited about rejoining the Ohio area. He enjoys the outdoors and he especially enjoys hiking; which he hopes to do more of.

Best wishes are extended to Chris and to his future endeavors.
Expression of Host Genes Associated with Inflammation, Scarring, and Fibrosis is Altered by Stressed Chlamydial Infection.

Regenia Phillips Campbell, Jennifer Kintner, Michelle Duffourc, Robert V. Schoborg

Presenting Author: Regenia Phillips Campbell

Exposure to environmental stressors such as cytokines, beta-lactam antibiotics, and nutrient deprivation induces chlamydiae to deviate from their normal developmental cycle in vitro. Data from clinical samples and animal models suggest these stressors may also alter the course of infection in vivo. Stressed chlamydiae alter host cell responses including apoptosis and may contribute to treatment failure, a growing concern in the clinical setting. We hypothesize stressed chlamydiae may alter expression of host genes associated with the inflammation, scarring, and fibrosis observed in some patients. To test this hypothesis, C. muridarum-infected murine oviduct epithelial cells were exposed to either amoxicillin to induce chlamydial stress or diluent controls. These data suggest chlamydia's highly expressed in stressed infections than in inflammation, and wound healing were less predicted to be involved in immune response, fibrosis observed in some patients. To test this hypothesis, C. muridarum-infected murine oviduct epithelial cells were exposed to either amoxicillin to induce chlamydial stress or diluent controls. These data suggest chlamydia's highly expressed in stressed infections than in inflammation, and wound healing were less predicted to be involved in immune response, inflammation, and wound healing were less highly expressed in stressed infections than in diluent controls. These data suggest chlamydia's stress response may allow the bacteria to evade the host immune response while inflicting cumulative histological damage.

Co-infection of BALB/c mice with Chlamydia muridarum and Herpes Simplex Virus (HSV) alters HSV pathogenesis.

Slade, Jessica, Hall, Jennifer V., Kintner, Jennifer, and Robert Schoborg

Presenting Author: Jessica Slade

Chlamydia trachomatis and Herpes Simplex Virus-2 (HSV-2), the leading bacterial and 2nd leading viral cause of sexually transmitted infections respectively, collectively caused more than 25 million new cases in the US in 2008. Co-infection of Chlamydia and HSV-2 has been reported in humans and demonstrated in vitro. In the current study, we present an in vivo model of C. muridarum/HSV-2 vaginal co-infection in BALB/c mice using 3 experimental scenarios: i) infection with 10^6 IFU C. muridarum (Cm) followed 3 days later by 5 x 10^3 PFU HSV-2 (Cm-3D-H3); ii) infection with HSV-2 followed 3 days later by C. muridarum infection (H3-3D-Cm); or iii) simultaneous infection with a combined inocula of C. muridarum and HSV-2 (Cm+H3). Mock, C. muridarum and HSV-2 singly-infected mice served as controls. After the first pathogen inoculation vaginal swabs were collected every 3 days for 21 days. Chlamydial titer assays and HSV-2 plaque assays were performed to monitor pathogen shedding. Sixty-six percent of mice infected with HSV-2 alone died by 15dpi; however, H3-3D-Cm mice died more rapidly (64% lethality by 12dpi) and shed more HSV-2 than HSV-2 singly-infected mice. Conversely, mice in Cm-D3-H3 and Cm+H3 groups showed significantly lower mortality (0 and 8%, respectively) and reduced viral shedding suggesting that prior chlamydial infection provided protection against HSV-induced fatal neurologic disease. Furthermore, C. muridarum pre-infection also protects animals infected with a higher HSV-2 inoculum (0% mortality in mice infected with Cm-3D-H10^5PFU versus 100% mortality in mice given 10^5PFU of HSV-2 alone). Mice co-infected with UV-inactivated C. muridarum prior to HSV-2 are also protected, albeit to a lesser degree than co-infection with live chlamydiae indicating that stimulation of TLRs by chlamydial products contributes to protection from HSV-stimulated death. Additionally, the protective effect is long-lasting, occurring even when HSV-2 infection followed C. muridarum infection by 9 days. We hypothesize that immune modulation or possibly epigenetic changes stimulated by C. muridarum infection decreases the detrimental actions of HSV-2 infection, thereby protecting mice from HSV-induced death.
EGGSHELL CALCIUM REGULATES EMBRYONIC GROWTH AND CALCIUM TRANSPORT IN AN OVIPAROUS SNAKE
Hannah F. Frye, James R. Stewart, Rebecca A. Pyles, Tom W. Ecay
Presenter Author: Rebecca A. Pyles

One hypothesis to explain the high incidence of independent evolutionary transitions from oviparity to viviparity among squamate (snake and lizard) reptile lineages proposed that embryonic development is independent of eggshell calcium. Recent research on embryonic calcium nutrition does not support this hypothesis as at least 25% of the calcium in hatchling oviparous squamates is extracted from the shell. An alternative hypothesis is that shell calcium supplements calcium from yolk in oviparous squamates, but is not obligatory for embryonic development. This hypothesis was tested by physically peeling the outer layers of the shell, and thus removing the calcium, early in development of Pantherophis guttatus (corn snake) eggs. The hypothesis was supported by experimental results showing that survivorship to hatching did not differ for peeled eggs compared to intact eggs. Yet hatchlings from peeled eggs were shorter (273.6 ± 3.4 vs. 261.0 ± 3.7 mm, p=0.0028, n=16), lighter (6.36 ±0.22 vs. 5.75 ± 0.23 g, p=0.0158, n=16), and had reduced calcium (40.8 ± 1.7 vs. 30.5 ± 1.8 mg, p<0.001, n=16), which could impact hatchling fitness in the wild. An additional hypothesis that embryos detect and respond to calcium availability was tested by assaying the two tissues involved in embryonic calcium acquisition for changes in calcium transport protein expression (by immunoblotting) following shell calcium removal. The yolk sac, involved in yolk calcium transport, showed no detectable change in the developmental expression of calbindin-D28K, a marker for calcium transport activity. However, the chorioallantois, involved in shell calcium transport, showed reduced calbindin-D28K expression relative to samples from intact eggs, suggesting eggshell calcium regulates chorioallantoic calcium transport.

The findings of this study suggest that evolution of viviparity is enhanced by a mechanism for detection and mobilization of calcium in oviparous embryos that also functions in the uterine environment.

Supported by an APS-IOSP fellowship to HF

THE PURINE NUCLEOTIDE CYCLE: A CARDIOPROTECTIVE PATHWAY INDUCED BY HIF-1A
Joe Wu, Cherie Bond, Ying Li, Gary Wright
Presenting Author: Joe Wu

Overexpression of hypoxia inducible factor 1α (HIF-1α) confers robust ischemic cardioprotection. Previously we showed that HIF-1α induces the ability to use fumarate as a terminal electron acceptor to sustain mitochondrial anaerobic electron transport chain (ETC) activity. The source of fumarate for ETC activity was established to be the purine nucleotide cycle (PNC). Here, we report that mRNA, protein, and activity of the rate-limiting enzyme in the PNC, AMP deaminase, is induced by HIF-1α. Thus, in addition to providing fumarate for anaerobic ETC activity, we hypothesized that induction of the PNC might serve as a mechanism that conserves the nucleotide pool during ischemia. The AMP that accumulates during ischemia can be metabolized by AMP deaminase to IMP, a membrane impermeable metabolite. Alternatively, AMP can be degraded to adenosine, which can diffuse into the interstitial space leading to a reduction of the cardiomyocyte's nucleotide pool. Consistent with our hypothesis, we show that HIF-1α-expressing hearts accumulate significantly less adenosine than wildtype hearts during ischemia. Collectively, our findings indicate that the PNC is a novel cardioprotective mechanism induced by HIF-1α that preserves ETC activity while conserving the nucleotide pool.

April 26-30, 2014, San Diego, CA

Ariella Jackson (Left) and Hannah Frye (Right) standing in front of the posters they presented at Experimental Biology 2014. Hannah has been working on an honors thesis project with Dr. Tom Ecay since the summer 2012. She has been supported for summer research by an NSF REU grant (2012) and an American Physiological Society summer fellowship in Integrative Organismal and Systems Physiology (2013). Ariella is an undergraduate from the University of Alabama-Birmingham and she was supported by the NSF REU grant for summer research in 2013.
Appalachian Student Research Forum
April 2014

FACULTY JUDGES
Many thanks to our faculty who served as Judges!

Dr. Alok Agrawal  Dr. Gregory Ordway
Dr. Jeffrey Ardell  Dr. Mitchell Robinson
Dr. Eric Beaumont  Dr. Robert Schoborg
Dr. Sharon Campbell  Dr. Krishna Singh
Dr. Michelle Chandle  Dr. Douglas Thewkes
Dr. J. Russ Hayman  Dr. Robert Wondergem
Dr. David Johnson  Dr. Gary Wright
Dr. Michael Kruppa

POSTERS ESENTATIONS
Undergraduate Students—Biomedical and Health Sciences, Group A
Husan Ahmad  Faculty Sponsor: Dr. Jeff Ardell
Timothy DiPeri  Faculty Sponsor: Dr. Gregory Ordway

Undergraduate Students—Biomedical and Health Sciences, Group B
Rebecca Howard  Faculty Sponsor: Dr. Mike Kruppa
John Magnson  Faculty Sponsor: Dr. Mike Kruppa
Hannah McNeill  Faculty Sponsor: Dr. Phillip Musich
Jonathan Millard  Faculty Sponsor: Dr. Krishna Singh
Megan Sears  Faculty Sponsor: Dr. David Johnson

Undergraduate Students—Natural Sciences
Hannah Frye  Faculty Sponsor: Dr. Tom Ecay

Graduate Students, Master’s Candidate—Biomedical Sciences, Group A
Martha Borketey  Faculty Sponsor: Dr. Sharon Campbell
Fidelis Ikweeme  Faculty Sponsor: Dr. Sharon Campbell

Graduate Students, Doctoral Candidates
Joe Wu  Faculty Sponsor: Dr. Gary Wright

Medical Residents Clinical Fellows & Postdoctoral Fellows, Group C
Aashish Morani  Faculty Sponsor: Dr. Sharon Campbell

Medical Students, Group A
Ben Cearlorc  Faculty Sponsor: Dr. Doug Thewke
Pharmacy Students, Group A
Chris Garst  Faculty Advisor: Dr. Doug Thewke

ORAL PRESENTATIONS
Doctoral Candidates, Medical Students, Pharmacy Students, and Post-Doctoral Fellows:
Regenia Campbell  Faculty Sponsor: Dr. Rob Schoborg
Laura Daniel  Faculty Sponsor: Dr. Krishna Singh
Jessica Slade  Faculty Sponsor: Dr. Rob Schoborg

(1st Place Winner)
MIXED TOCOTRIENOL DIET SUPPLEMENTATION ATTENUATES BEHAVIORAL, MORPHOLOGICAL AND BIOCHEMICAL ALTERATIONS ASSOCIATED WITH SOCIAL DEFEAT STRESS IN FISHER 344 RATS.

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Major depressive disorder (MDD) is a psychiatric illness with enormous economic burden both in the Unites States and globally. Current therapy for MDD aims at the central monoaminergic systems. These medications take time to reach its therapeutic potential and also have severe adverse effects. Therefore there is a need to search novel agents for MDD therapy. Oxidative stress has been implicated in MDD with enhanced levels of reactive oxygen species (ROS) followed by a decreased anti-oxidant enzyme levels in brain and circulation. The tocopherols and tocotrienols have been considered as natural anti-oxidants. Furthermore, supplementation with α-tocopherol attenuates MDD symptoms in clinical and pre-clinical studies. This study aimed at understanding the effects of dietary mixed tocotrienol (MT3) supplementation on the development of MDD symptoms using a behavioral paradigm of social defeat stress (SDS) in laboratory rats. Fisher 344 male rats (intruders) were subjected to the SDS with the Long Evans male rats (resident aggressors) for 10 consecutive days. The SDS procedure comprised of an initial direct contact (maximum of 10 min; Phase 1) followed by the phase of indirect (visual and olfactory) contact (60 min; Phase 2). In order to test the development of anhedonia, sucrose preference test (SPT; d5, d10) and social interaction tests (SIT; d10) were performed. Following urine and blood collection, animals were euthanized (d14) and their brains harvested. Biochemical estimations of urinary nitric oxide (uNO) were determined. Additionally, morphological estimations of the hippocampal CA3 regions were performed using the cresyl violet (CV) stain. In order to demonstrate the production of superoxide anion in the hippocampal regions, dihydroethidium bromide (DHEB) staining was performed. Rats treated with normal diet-SDS (ND/SDS) preferred water over sucrose, indicating anhedonia. They also spent more time in the avoidance zone than the interaction zone during the SIT, which is an indication of depressive behavior. However, this effect was not significant. A significant enhancement in the levels of uNO and relative fluorescence intensity of DHEB staining in the hippocampal CA3 region was noted for ND/SDS treated rats compared to ND/CTRL group of rats. The derangement in the hippocampal pyramidal cells was observed in ND/SDS rats, which was not seen in ND/CTRL rats. These results collectively show that social defeat stress inflicts depressive behavior with morphological alteration in the hippocampus. Also, SDS produces increases in the levels of ROS in the brain and urine. MT3 diet supplementation reversed the observed behavioral, morphological and biochemical alterations induced by the social defeat sessions. MT3 diet produces preventive effects on behavioral and morphological abnormalities induced by SDS in fisher 344 rats, the mechanism of which may involve its anti-oxidant properties. More studies are required to determine the exact mechanisms of actions of MT3.
Abstracts

First Place Winner, Medical Students, Group A

EFFECT OF OXIDIZED LOW-DENSITY LIPOPROTEINS ON MODULATION OF OSTEOGENIC DIFFERENTIATION OF VASCULAR CELLS BY THE TYPE-2 CANNABINOID RECEPTOR (CB2)

Ben Cearlock, Kaitlyn Hinshaw, Zachary Lahr and Douglas Thewke, Department of Biomedical Sciences, Quillen College of Medicine, East Tennessee State University

Introduction: Atherosclerosis is an inflammatory disease characterized by formation of lipid-rich lesions within arterial walls which, in more advanced stages, become calcified. Lesion calcification is associated with increased vulnerability to rupture and the clinical consequences of atherosclerosis, myocardial infarction and stroke. Calcification is a cell-mediated process similar to bone remodeling during which the formation of osteoblast-like cells outpaces that of osteoclast-like cells. Previously, we found that the type-2 cannabinoid receptor (CB2) modulates the cellular composition of atherosclerotic lesions in mice, and in vitro, modifies the response of macrophages to oxidized lipids (such as oxLDL). In vitro, CB2 promotes osteoblastogenesis and inhibits osteoclastogenesis. Since oxLDL accumulates within lesions and calcification occurs in close proximity with lipids, we hypothesize that osteogenic processes within lesions are, in part, modulated by CB2 signaling and oxLDL. To begin to test this hypothesis we determined the effects of oxLDL on CB2 modulation of osteogenesis using cell lines representative of cell types within atherosclerotic lesions.

Methods: Raw264.7 cells, a murine monocytic cell line which undergoes osteoclastogenesis in response to Receptor Activator of Nuclear factor Kappa B Ligand (RANKL), were cultured in a media containing RANKL, varying concentrations of Win55,212-2 (a synthetic CB1/CB2 agonist), and oxLDL. Osteoclastogenesis was assayed by measuring tartrate-resistant acid phosphatase (TRAP) activity. MOVAS-1 cells, a murine VSMC cell line which spontaneously differentiates into osteoblasts, were cultured in media containing varying concentrations of Win55,212-2 and oxLDL. Osteoblastogenesis was evaluated by assaying for alkaline phosphatase (ALP) activity and by Alizarin Red S staining for calcium deposition.

Results: Raw264.7 cells treated with Win55,212-2 or oxLDL displayed a dose-dependent decrease in RANKL-induced TRAP activity. Co-treatment with both oxLDL and Win55,212-2 further reduced RANKL-stimulated TRAP activity. Osteoblastic differentiation of MOVAS-1 cells, as determined by ALP activity, was enhanced by Win55,212-2 treatment, while oxLDL supplementation alone had little effect. When supplemented together, oxLDL inhibited the ability of Win55,212-2 to stimulate osteoblastogenesis. However, at the concentrations tested, oxLDL produced no discernable effects on Win55,212-2 stimulated calcium deposition.

Conclusions: These results show that oxLDL enhances the inhibitory effect of CB2 activation on osteoclastogenesis in vitro. However, the effect of oxLDL on CB2 activation of osteoblastogenesis is less clear, and requires further investigation. This work provides new insight into potential mechanisms affecting lesion calcification. Understanding the role of CB2 and oxLDL during lesion calcification may lead to the development of novel drugs to treat heart disease.

Appalachian Student Research Forum—2014

First Place Winner, Medical Students, Group A

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EXPRESSION OF HOST GENES ASSOCIATED WITH INFLAMMATION, SCARRING, AND FIBROSIS IS ALTERED BY STRESSED CHLAMYDIAL INFECTION.

Regenia Phillips Campbell, Jennifer Kintner, Michelle Duffourc, Robert V. Schoborg, Quillen College of Medicine

First Place Winner, Doctoral Candidate

Oral Presentation

EXPRESSION OF HOST GENES ASSOCIATED WITH INFLAMMATION, SCARRING, AND FIBROSIS IS ALTERED BY STRESSED CHLAMYDIAL INFECTION.

Regenia Phillips Campbell, Jennifer Kintner, Michelle Duffourc, Robert V. Schoborg, Quillen College of Medicine

Exposure to environmental stressors such as cytokines, beta-lactam antibiotics, and nutrient deprivation induces chlamydiae to deviate from their normal developmental cycle in vitro. Data from clinical samples and animal models suggest these stressors may also alter the course of infection in vivo. Stressed chlamydiae alter host cell responses including apoptosis and may contribute to treatment failure, a growing concern in the clinical setting. We hypothesize stressed chlamydiae may alter expression of host genes associated with the inflammation, scarring, and fibrosis observed in some patients. To test this hypothesis, C. muridarum infected murine oviduct epithelial cells were exposed to either amoxicillin to induce chlamydial stress or diluent as a mock control. RNA collected 24 and 48 hours after stressor exposure was analyzed by microarray for host gene expression. Quantitative PCR validates expression of Mmp3, a gene predicted to play a role in collagen
Regenia Phillips Campbell (Cont’d.)

catabolism, was increased 109 fold in infected samples compared to mock-infected controls, but only 42 fold in moxicillin-exposed infections. Similarly, expression of predicted cytokine Ifn1b increased 661 fold in infected samples, but only 23 fold in samples exposed to the amoxicillin stressor. Further, Gp49a, Lirnb4, and Krt16, genes predicted to be involved in immune response, inflammation, and wound healing were less highly expressed in stressed infections than in diluent controls. These data suggest chlamydia’s stress response may allow the bacteria to evade the host immune response while eliciting cumulative histological damage.

INDUCTION OF HYPOXANTHINE PHOSPHORIBOSYL TRANSFERASE BY HIF-1α: IMPLICATIONS IN PROTECTION AGAINST ISCHEMIA-REPERFUSION INJURY

Joe Wu, Cherie Bond, Ying Li, and Gary Wright
Department of Biomedical Sciences

Ischemia diminishes O2 delivery to the heart. This suppresses ATP synthesis through oxidative phosphorylation. While glycolysis generates ATP anaerobically, it does not provide adequate ATP to meet the heart’s energy demands. Thus, the degradation of ATP occurs, which ultimately leads to the accumulation of nucleobase hypoxanthine in the ischemic heart. Hypoxanthine can be a beneficial or harmful metabolite in the heart upon reperfusion from ischemic stress. Should hypoxanthine be taken up by the salvage enzyme hypoxanthine phosphoribosyl transferase (HPRT), the resynthesis of ATP can occur. Alternatively, upon reperfusion, the availability of oxygen allows xanthine oxidase to convert hypoxanthine to xanthine. This reaction leads to a burst of reactive oxygen species (ROS) which induces reperfusion injury. Previously, we observed that mouse hearts that overexpress hypoxia inducible factor 1α (HIF-1α) exhibited a tendency to recover a higher percentage of their pre-ischemic ATP. In addition, HIF-1α conferred robust protection against reperfusion injury in mouse hearts. Further studies led us to discover that HPRT was induced by HIF-1α on the transcriptional and protein level. Our results may indicate that HPRT is a crucial part of the HIF-1α mediated adaptive response that aids in the resynthesis of ATP upon reperfusion from ischemia. In addition, as HPRT will compete with xanthine oxidase for hypoxanthine, the induction of this pathway by HIF-1α may help attenuate ROS production during reperfusion, thus limiting injury. Future studies will 1) confirm that HPRT contributes significantly to the regeneration of ATP during reperfusion and 2) establish that HIF-1α reduces ROS formation during reperfusion and that this effect is attributed to its upregulation of HPRT.

EFFECTS OF GAMMA TOCOPHEROL AND GAMMA TOCOTRIENOL ON FATTY ACIDS METABOLITES IN COLORECTAL CANCER

Martha A Borketey, Stacy D Brown, Sharon E Campbell
Department of Chemistry, Department of Pharmacy, Gatton College of Pharmacy, Department of Biomedical Sciences, James H. Quillen College of Medicine

Purpose: Colorectal cancer is ranked as the third most common cancer in both male and female in the United States. Lipooxygenase (LOX) metabolites have been associated with proliferation of colorectal cancer. A decrease in the level of 13 HODE (a 15-Lox-1 metabolite) in colorectal cancer enhances the growth of the cancerous cells. Increase in 13-HODE induces growth arrest and apoptosis in colorectal cancer cells. 12-HETE and 5-HETE plays an important role in the progression of colorectal cancer through inflammatory response pathways. 15-HETE reduces cell proliferation and causes apoptosis in colorectal cancer cells. Gamma tocopherol (GT) and gamma tocotrienol (GT3) may reduce proliferation of colon cancer by regulating the production of LOX metabolites. We hereby studied the effects of gamma tocopherol and gamma tocotrienol in the production of LOX metabolites.

Method: LOX metabolites, 13-HODE, 15-HETE, 12-HETE and 5-HETE were extracted from HCT-116 cells after 24 hour treatment with GT and GT3 and quantified by liquid chromatography-mass spectrometry (LC/MS). The level of each metabolite in the treated cells was compared to the untreated cells.

Results: Treatment of HCT-116 colon cancer cells with GT or GT3 resulted in the elevation of 13-HODE, 15-HETE, 12-HETE and 5-HETE were depressed with treatment by both GT and GT3. Conclusion: The results obtained here suggest that GT and GT3 may reduce proliferation of colorectal cancer cells by up-regulation of 13-HODE through enhancement of apoptosis. It is also possible that GT and GT3 may reduce cell proliferation through the down regulation of 12-HETE and 5-HETE by modulating inflammation. Further investigations are required to determine the role of GT and GT3 and the modulation of fatty acid metabolites in colorectal cancer.
Colon cancer is the third most common cancer worldwide and ranks second as the leading cause of cancer-related deaths. Despite advances in cancer therapeutics, colon cancer is still on the rise. Gamma tocotrienol (GT3) has demonstrated not only anti-oxidant properties but also anti-cancer properties. GT3 induces ER stress and regulates protein translation, a vital process for cancer cell immortality. Stress may result in the inhibition of protein translation through down-regulation of p-p38 and p-ERK MAP Kinases. This leads to a hypo-phosphorylation of downstream translation initiation factors EIF4G and EIF4E and arrest of protein synthesis. GT3 induces ER stress in a dose-dependent fashion by down-regulating p-p38 and p-ERK MAP kinases. Pro-apoptotic proteins are also up-regulated during ER stress, with an increase in poly ADP ribose polymerase protein cleavage resulting in controlled cell death. Temsirolimus (TEM), an inhibitor of the mammalian target of rapamycin (mTOR) is currently being explored in clinical trials as a potent therapeutic agent for colorectal cancer. The mTOR pathway feeds into several metabolic processes including protein translation by regulating phosphorylation of translation initiation factors EIF4G and EIF4E. The central hypothesis of this study is that combined treatment of colon cancer cells with TEM and GT3 would be efficacious in preventing uncontrolled cell division, inhibition of apoptotic proteins, and continuous protein translation machinery. Combination therapy with TEM and GT3 would also be beneficial by achieving a potential dose reduction in TEM which would reduce adverse effects associated with potent chemotherapeutics. Our results show a reduction in the dose required for TEM and GT3 in achieving a fifty percent growth inhibition on HCT-116 colon cancer cell line when used in combination versus individually. Overall, synergistic inhibition based on the Chou-Talalay method was also observed at levels as low as twenty-five percent growth inhibition. Western blot analysis has shown a down-regulation of p-p38 and p-mTOR in HCT-116 cells treated with TEM and GT3 combined at 5µM and 25µM, respectively suggesting a promising cross-talk in controlling protein translation via differing mechanisms.

Background and Significance: Chlamydia trachomatis is the most common sexually transmitted bacterial disease agent worldwide and can co-infect with other genital pathogens, like Herpes Simplex Virus (HSV). Chlamydiae are obligate intracellular pathogens that exhibit a unique biphasic developmental cycle, in which infectious elementary bodies (EB) are internalized into a host cell within a membrane-bound inclusion. Once in the inclusion, the EB transform to noninfectious, replicable reticulate bodies (RB), which replicate and then convert back into EB, which are released and can infect new host cells. HSV super-infection of a C. trachomatis serovar E (CtE) infected host genital epithelial cell induces the chlamydiae to leave the normal developmental cycle and enter a viable but non-infectious (persistent/stressed) state by a mechanism that requires HSV glycoprotein D/host nectin-1 protein interaction. HSV/host cell binding also activates JAK, JNK, and PI3K, suggesting these signaling pathways may be involved in HSV-mediated, persistence induction. Objectives: To test the hypotheses that: i) JAK, JNK, and/or PI3K inhibition aborts the normal chlamydial developmental cycle; and ii) host nectin-1 is required for normal chlamydial development in the absence of HSV co-infection. Methods: HeLa (human genital epithelial cell) monolayers were mock- or CtE-infected for 23 hours (h) before addition of JAK, JNK, or PI3K inhibitors. After a 21h incubation, cultures were chlamydial elementary bodies (EB). In other experiments, host nectin-1 specific shRNA-expressing plasmids were transfected into host HeLa cells to knockdown nectin-1 expression. The nectin-1 knockout cells were then chlamydia infected and assayed for inclusion development and infectious EB production. Results and Conclusions: All of the inhibitors tested reduced the production of infectious chlamydial progeny. Interestingly, nectin-1 knockout cells produce less infectious chlamydial organisms after infection than do nectin-1 replete control host cells. These data indicate that JAK, JNK, and/or PI3K and host cellular nectin-1 are required for normal chlamydial development.
EFICIENCY OF ATM MODULATES INFLAMMATORY RESPONSE AND APOPTOTIC SIGNALING IN THE HEART FOLLOWING MYOCARDIAL INFARCTION

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Background: Ataxia-telangiectasia (A-T) is a rare autosomal recessive genetic disease caused by a mutation in the gene encoding for Ataxia Telangiectasia Mutated Kinase (ATM). Individuals with this disease have compromised immunity and carriers of the mutation have an increased risk of developing ischemic heart disease. Previously, using ATM heterozygous knockout (hKO) and wild-type (WT) mice, we have shown that deficiency of ATM attenuates LV dysfunction and dilatation 7 days post myocardial infarction (MI). ATM deficiency also resulted in increased cardiac cell apoptosis in the border region 7 days post-MI. Changes in inflammatory response are suggested to modulate myocardial remodeling post-MI. Proper timing of this response is a critical event in the healing process post-MI. The objective of this study was to investigate the role of ATM in the induction of inflammatory response, and activation of survival signaling molecules in the heart post-MI.

Methods: MI was performed in hKO and WT mice by ligating the left anterior descending artery. Cellular and biochemical parameters of the heart were measured 1 and 3 days post-MI. Apoptosis was measured using TUNEL assay. Migration of neutrophils and macrophages into the heart was measured using immunohistochemical staining. Protein levels of transforming growth factor-β1 (TGF-β1; an anti-inflammatory protein), and phosphorylation of apoptosis-related proteins, Akt and glycogen synthase kinase-3 beta (GSK-3β), were examined using western blots.

Results: The number of neutrophils and macrophages was significantly (p<0.05) lower in the infarct region of hKO mice when compared to WT mice 1 day post-MI. However, there was no difference between the two genotypes 3 days post-MI. Although expression of TGF-β1 was not detected in the sham groups or in the infarct regions 1 day post-MI, it was detected in the infarct regions 3 days post-MI. Interestingly, the expression of TGF-β1 was found to be significantly lower (p<0.05) in hKO mice when compared to WT mice. The number of apoptotic cells was higher in the infarct region of hKO hearts (p<0.05) versus WT 1 and 3 days post-MI. Phosphorylation of Akt and GSK-3β was significantly higher (p<0.05) in the infarct region of WT mice when compared to hKO mice 1 day post-MI. However, phosphorylation of Akt and GSK-3β was not different between the two genotypes 3 days post-MI. Conclusion: Deficiency of ATM induces a delayed inflammatory response, while down-regulating the survival signaling pathway/s. Further investigations are needed to determine how the changes in inflammatory and survival signals affect myocardial remodeling late post-MI during ATM deficiency.

EXTRACTION OF BIOMARKERS OF ATHEROSCLEROSIS IN AORTIC TISSUE AND PLASMA

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Introduction: Atherosclerosis is a disease characterized by plaque formation due to an accumulation of fat, cholesterol, and immune cells in the walls of arteries. Plaques can become unstable and rupture leading to a possible heart attack or stroke. Macrophages are one of the immune cells found to play a role in plaque destabilization and rupture. CB-2 cannabionoid receptors are hypothesized to inhibit immune activity and may provide benefit in the treatment or possibly prevention of atherosclerosis. Endogenous cannabinoids, arachidonylethanolamide (AEA) and 2-arachidonyl glycerol (2-AG) have been found to have activity on CB-1 and CB-2 receptors throughout the body. The role of these compounds and their action on CB-2 receptors is not completely understood. Purpose: To develop an extraction method for AEA and 2-AG from plasma and aortic tissue samples and an LC-MS/MS assay for quantification of these compounds to help understand the role of endocannabinoids and CB-2...
Abstracts

Christopher Garst (Cont’d)

receptors in atherosclerosis. Method: Tissue samples were prepared by obtaining the weight of the aortic tissue and adding an equivalent amount in mL of MES Buffered Saline. The tissue was homogenized by hand in a silanized glass homogenizer and a 500μL aliquot of the mixture was used for the extraction process. Plasma samples were prepared by precipitating protein with 1mL of ice cold acetone and obtaining a 500μL aliquot of the sample for extraction. Liquid-liquid extraction (LLE) of tissue or plasma was carried out by adding toluene in a 1:2 ratio to each aliquot in an LC-MS/MS assay for quantification of these compounds to help understand the role of endocannabinoids and CB-2 receptors in atherosclerosis. Method: Tissue samples were prepared by obtaining the weight of the aortic tissue and adding an equivalent amount in mL of MES Buffered Saline. The tissue was homogenized by hand in a silanized glass homogenizer and a 500μL aliquot of the mixture was used for the extraction process. Plasma samples were prepared by precipitating protein with 1mL of ice cold acetone and obtaining a 500μL aliquot of the sample for extraction. Liquid-liquid extraction (LLE) of tissue or plasma was carried out by adding toluene in a 1:2 ratio to each aliquot in microcentrifuge tubes. Samples were then vortexed for 30 seconds and then centrifuged (10000 rpm, 5min, 25°C). The supernatants were then transferred to glass tubes and evaporated to dryness at 35°C under nitrogen. Samples were reconstituted with 100μL of acetonitrile and analyzed using a direct LC-MS/MS assay operating in positive electrospray (ESI) mode. Results: The aforementioned extraction process proved superior to several other methods with regards to linearity and analyte recovery. Other extraction techniques that were screened for this project included solid phase extraction (SPE, with C8 and mixed-mode HLB cartridges), solid liquid extraction (SLE), polymixin B extraction, and other LLE. The linear range of the final assay was 0.5 – 10 mcg/ml for both AEA and 2AG. Addition of deuterium-labeled (d8) analogs of each analyte helped improve quantification of the analytes from these complex matrices. Additionally, utilizing the direct MS/MS channels corresponding to the analytes’[M+H+] ion helped increase sensitivity and reduce noise for the analytical measurements. Conclusion: This assay can be applied toward the measurement of endogenous cannabinoids, AEA and 2AG, in aortic tissue and plasma. The technique will be utilized in an investigation of the effects of atherogenic high fat diet on wild type and CB2 -/- mice

EGGSHELL CALCIUM REGULATES EMBRYONIC GROWTH AND CALCIUM TRANSPORT IN AN OVIPAROUS SNAKE

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One hypothesis to explain the high incidence of independent evolutionary transitions from oviparity to viviparity among squamate (snake and lizard) reptile lineages proposed that embryonic development is independent of eggshell calcium. Recent research on embryonic calcium nutrition does not support this hypothesis as at least 25% of the calcium in hatching oviparous squamates is extracted from the shell. An alternative hypothesis is that shell calcium supplements calcium from yolk in oviparous squamates, but is not obligatory for embryonic development. This hypothesis was tested by physically peeling the outer layers of the shell, and thus removing the calcium, early in development of Pantherophis guttatus (corn snake) eggs. The hypothesis was supported by experimental results showing that survivorship to hatching did not differ for peeled eggs compared to intact eggs. Yet hatchlings from peeled eggs were shorter (273.6 ± 3.4 vs. 261.0 ± 3.7 mm, p=0.0028, n=16), lighter (6.36 ±0.22 vs. 5.75 ± 0.23 g, p=0.0158, n=16), and had reduced calcium (40.8 ± 1.7 vs. 30.5 ± 1.8 mg, p<0.001, n=16), which could impact hatching fitness in the wild. An additional hypothesis that embryos detect and respond to calcium availability was tested by assaying the two tissues involved in embryonic calcium acquisition for changes in calcium transport protein expression (by immunoblotting) following shell calcium removal. The yolk sac, involved in yolk calcium transport, showed no detectable change in the developmental expression of calbindin-D28K, a marker for calcium transport activity. However, the chorioallantois, involved in shell calcium transport, showed reduced calbindin-D28K expression relative to samples from intact eggs, suggesting eggshell calcium regulates chorioallantoic calcium transport. The findings of this study suggest that evolution of viviparity is enhanced by a mechanism for detection and mobilization of calcium in oviparous embryos that also functions in the uterine environment. (Supported by an APS-IOSP fellowship to HF)
QUANTIFICATION OF XPA DNA REPAIR PROTEIN IN CANCER, NORMAL, AND PROGEROID CELLS

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The efficient repair of DNA damage appears to be an important factor in the aging process. Xeroderma pigmentosum group A (XPA) is a protein normally involved in nucleotide excision repair (NER) of damaged bases in DNA. We have shown that XPA protein normally is localized in the cytoplasm of cells and translocates to the nucleus in response to DNA damage caused by ultraviolet light or the chemotherapy agent cisplatin. Hutchinson-Gilford progeria syndrome (HGPS) patients suffer from rapid premature aging, with an average life expectancy of only ~13 years. Previous studies observed a deficiency in the NER process in fibroblast cells isolated from HGPS patients. These and related studies also found that more of the XPA in HGPS cells was sequestered in the nucleus of the cell than being free in the cytoplasm. This study will investigate whether there are quantitative differences in the cellular levels of XPA between cancer cells and normal and HGPS fibroblasts. It is hypothesized that XPA levels will be higher in the rapidly growing cancer cells while being reduced in HGPS and normal human fibroblasts. Total cell lysates were prepared from lung cancer and bone cancer cells and HGPS and normal human fibroblasts that were propagated in cell culture. Cancer cells were chosen due to their rapid growth and efficient NER. Normal human fibroblasts were chosen as a control because the HGPS cells are normal fibroblast cells except that they show premature aging. The levels of individual proteins were quantified using SDS-polyacrylamide gel electrophoresis followed by western blotting. The amount of XPA was normalized to the level of β-actin or glyceraldehyde 3-phosphate dehydrogenase; the latter were used as protein loading controls. Normalized XPA levels then were compared across the various cell types and ages. The data show that as normal human fibroblasts aged the amounts of XPA decreased. Additionally the levels of XPA in cancer cells were about ten-times higher than in normal and progeroid cells. The results for the aging progeroid cells indicate that the levels of XPA increase as the HGPS cells age in culture. This observed increase of XPA in HGPS is contrary to the observed decrease of XPA found in the normal fibroblast control group. However, an increase in XPA may correlate with the previously observed sequestration of most of the XPA to sites of DNA damage in the chromatin of aging HGPS cells. Also, our observed increase in total XPA protein is small relative to the increased chromatin sequestration, and, thus, consistent with the reduced NER activity observed earlier. These data are being confirmed by looking at XPA levels in other HGPS and normal fibroblasts including those isolated from HGPS patients and their parents as a check for natural population variability.
EXTRACELLULAR UBIQUITIN REGULATES EXPRESSION OF β3 INTEGRINS AND VEGF-A VIA THE INVOLVEMENT OF CXCR4 IN CARDIAC FIBROBLASTS

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Background: Recent research has elucidated the pleiotropic physiological effects of ubiquitin (UB) in the body. The most well established function of UB is the marking of faulty or excessive intracellular proteins for degradation via the UB proteasome pathway. Contemporary research has explored the role of extracellular UB in modulation of immune response, cell migration, apoptosis, and angiogenesis. Our lab has shown that stimulation of b-adrenergic receptors (b-AR) increases extracellular levels of UB, and extracellular UB inhibits b-AR-stimulated apoptosis and fibrosis in the heart. This study tested the hypothesis that extracellular UB, acting via C-X-C chemokine receptor 4 (CXCR4), modulates expression of β3 integrins (transmembrane receptor protein involved in cell migration) and vascular endothelial growth factor-A (VEGF-A; angiogenic protein involved in tissue repair) in cardiac fibroblasts. Methods: Serum starved confluent cultures of adult rat cardiac fibroblasts were treated with UB (10 µg/ml) for 24 h. To investigate the role of CXCR-4, the cells were pretreated with AMD3100 (CXCR4 antagonist; 10 µM) for 30 min followed by treatment with UB for 24 h. To confirm the role of CXCR-4, the cells were treated with UB mutants (10 µg/ml; mUB V70 and mUB F4; unable to interact with CXCR4) for 24 h. Cell lysates were analyzed by western blots using anti-β3 integrin or VEGF-A antibodies. Results: UB treatment significantly decreased the expression of β3 integrins when compared to untreated controls (CTL; p<0.05 vs CTL). AMD3100 alone had no significant effect on β3 integrin expression. However, it almost completely inhibited UB-mediated decrease in the expression of β3 integrin (CTL: 1.00±0.01; UB: 0.45±0.09; AMD+UB: 0.99±0.22; AMD: 0.97±0.18; *p<0.05 vs CTL; n = 5). Treatment with two UB mutants had no effect on β3 integrin expression. UB treatment significantly increased VEGF-A expression (p<0.05 vs CTL). AMD3100 alone had no significant effect on VEGF-A expression. However, it almost completely inhibited UB-mediated increases in VEGF-A expression (CTL: 1.00±0.01; UB: 3.22±0.6; AMD+UB: 1.49±0.3, AMD: 1.15±0.1; *p<0.05 vs CTL; n = 5). Treatment of cells with mutated UBs had no effect on VEGF-A expression.

Summary: Extracellular UB, most likely acting via CXCR4, affects expression of β3 integrins and VEGF-A. Future explorations of the constituents of this pathway in heart cells could potentially lead to preventative therapies/treatments for cardiovascular disease.

ISOLATION OF EXTRACYTOSOLIC VESICLES FROM CANDIDA ALBICANS: A MECHANISM OF CELLULAR COMMUNICATION?

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The opportunistic fungal pathogen Candida albicans is the most commonly isolated organism identified in critical care facilities. This pathogen has several mechanisms pertaining to its virulence including the ability to undergo a reversible morphological change between yeast and hyphal forms. How C. albicans controls this switch between morphologies has been under investigation by a number of laboratories including ours. This morphological switch is controlled by a number of factors including pH, presence of serum, temperature, cell density, and more recently quorum sensing (QS). QS in C. albicans is regulated by the isoprenoid molecule trans,trans-farnesol. QS is tied to cell density in that high cell densities tend to have high levels of farnesol and are predominantly yeast in morphology, while lower cell densities can result in the shift to a hyphal morphology at physiologic temperature (37°C). Though farnesol has been purified from supernatants of yeast cells, it is not yet known if farnesol is free in the environment or part of a complex of molecules such as extracytosolic vesicles(EVs) which are secreted into the environment. We hypothesize that farnesol is present in EVs and that these vesicles are the vehicles for message delivery during QS. For EVs isolation, wild type C. albicans strain SC5314 was grown for 48 hours in either 5 mLs Medium 199, pH3.5 at 30°C or in 5mL Yeast Peptone Dextrose (YPD) medium at 30°C to obtain material from the yeast form. To obtain EVs from the hyphal form, SC5314 was grown for 48 hours in 5mL Medium 199, pH 7.5 at 37°C. The cells were separated from the media by filtration through a 0.45µm filter and the filtered medium was saved for EVs isolation. Briefly 1mL of filtered medium was mixed with 0.5mL Invitrogen Total Exosome Isolation reagent and incubated overnight at 4°C. The EVs were harvested by centrifugation at 10,000 x g and the pellet suspended in 25µL 1x PBS, pH 7.4. EVs were visualized by scanning electron microscopy and shown to have variable sizes ranging from 50nm to 200nm which fits the known sizes for these types of particles. To ascertain whether the EVs had QS activity, we incubated 5x10⁶cells/mL in Medium 199, pH 7.5 at 37°C with 300µM farnesol, without farnesol, and with 5µL EV added from either yeast or hyphal extracts.
for 2 hours. Under these conditions, samples that had no farnesol filamented with >90% appearing as hyphae, while samples with farnesol showed strong impairment of filamentation (less than 30% hyphal cells), and those that contained added EVs showed at least 50% impairment of filamentation, thus indicating that there is some QS effect associated with EVs secreted from *C. albicans* cells. These results support our belief that EVs serve as the communication mechanism between *C. albicans* cells. Characterizing the role EVs play in QS and pathogenesis could potentially lead to development of new antifungals that mimic EVs activities as a way to control infections by *C. albicans*.

**RTA3 AND GNP3 HAVE A POTENTIAL ROLE IN THE QUORUM SENSING RESPONSE OF CANDIDA ALBICANS**

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Quorum sensing (QS) is a mechanism of communication between cells to coordinate biological activities. In the opportunistic fungal pathogen *Candida albicans* QS is important for regulating the organism’s ability to change morphology from a yeast form to a hyphal form. Previously our laboratory identified several genes involved in polymicrobial communication between *C. albicans* and three different bacterial species. We hypothesize that interspecies communication and QS are utilizing the same genetic pathways to control *C. albicans* morphological behavior. In this study we have examined if the transport genes *RTA3* or *GNP3* are involved in QS. To test whether these two genes participate in QS, we performed a filamentation assay. *C. albicans* wild type strain SC3514 along with CHK21, and the haploinsufficient strains *hRTA3* and *hGNP3* were grown overnight in YPD at 30°C. The cells were washed, and diluted to 5 x 10⁵ cells/mL in Medium 199, pH 7.5 (M199) with or without the presence of 300μM tran,trans-farnesol. The cells were then grown at 37°C for 2.5 hr to allow for filamentation. The samples were then harvested and morphological characteristics determined. It was found that the SC5314 filamented normally at 37°C with >90% hyphae produced in the absence of farnesol but this was reduced to less than 45% in the presence of farnesol. The CHK21 strain is a histidine kinase mutant that does not respond to farnesol and mirrored the wild type in the absence of farnesol but >78% of the cells were filamenting in the presence of farnesol. Both the *hRTA3* and *hGNP3* strains filamented normally in the absence of farnesol, but in the presence of farnesol there was a slightly increased presence of hyphal filaments (around 50-55% filamentation for both strains) suggesting that both mutants are mildly impaired in their response to the QS molecule. As with the filamentation assay, cells were grown overnight at 30°C in YPD, harvested, washed and serially diluted from 10⁷ to 10⁴ cells/mL. One hundred microliters of sample from each dilution was inoculated into a 96 well plate and allowed to adhere to the surface of the plate for 90 min. The supernatants and unbound cells were removed by washing. Fresh M199, pH 7.5 with or without farnesol was added to each well. The plates were incubated for 48 hr after which the supernatants were removed and biofilms washed and stained. In the absence of farnesol the SC5314 and CHK21 strains formed robust biofilms, but in the presence of farnesol biofilm growth was reduced substantially with the SC5314 by 80% but only 30% with the CHK21 strain. With the *hRTA3* and *hGNP3* strains biofilm formation was far less apparent at lower cell densities without farnesol; when farnesol was present there was no increase in reduced biofilm formation. These results suggest that the *hRTA3* and *hGNP3* are naturally impaired in biofilm formation but not in filamentation suggesting a separation of QS function from biofilm formation.